August 29, 1956

Dear Dr. Sabin:

Thank you for your letter of August 27. I am sorry you have no longer maintained the pleuropneumonia collection, but will write to the people you suggested.

If I was not clear about the relationships of protoplasts to L-forms that was only indicative of my current understanding of them. It is obvious to me now that my protoplasts are Dienes' initial large bodies. In liquid medium, these enlarge but do not proliferate. The high sucrose content stabilizes the protoplasts against lysis, so they can maintain their metabolic activity, and ability to revert to bacteria, for a long time. With E. coli, in the absence of sucrose, you would get very few protoplasts; with Proteus, a few percent of them survive. Dienes' statement that (under his conditions) all the viable cells formed large bodies is undoubtedly based on the lytic disappearance of the majority of the cells.

Now, I am trying to go through Dienes' procedures, with the hope that the understanding of the osmotic fragility of protoplasts may help to put the study of L forms on a more exact quantitative basis. I am learning more every day, but still feel very far from a grasp on the whole problem. In soft agar, the protoplasts of Proteus, and to a lesser extent of E. coli K-12, are capable of proliferating to form "colonies" which contain up to about 1000 spherical bodies and considerable granular debris. As far as I can tell so far, from limited slide culture observations, this proliferation is by budding from the spheres. But to date, I have had very limited success in further passaging of these colonies and none at all in "plating them out". However, I have not yet run the gamut of all the published recipes.

Yours sincerely,

Joshua Lederberg